

Studying biomarkers reflecting the adverse outcome pathways from exposures to diseases using molecular epidemiology

Example of the molecular epidemiology protocol developed as part of the Concerted Uranium Research in Europe (CURE) project:

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Limitations of classical epidemiological studies, and complementary insights from biology

Statistical power and other issues make it more difficult to directly quantify precisely cancer risk in humans at organ doses of <100 mGy* (NRC 2006)

Classical epidemiology does not allow

- to test for biological plausibility
- to investigate mechanisms

➔ Only biology/molecular epidemiology can investigate induced biological molecular initiating events and molecular key events related to the adverse outcome (disease) to support risk extrapolation to very low dose.

*depending on the cancer and population

Example from the CURE Project «Concerted Uranium Research in Europe»



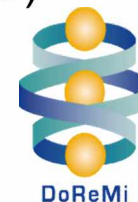
Aim

To elaborate a collaborative research project, integrating epidemiology, biology/toxicology and dosimetry to improve risk estimation of uranium contamination in occupational context

Included a **feasibility study for a molecular epidemiology** approach (measuring **biomarkers** for integration into the risk assessment process)

Characteristics

- **9 Participants** (IRSN, BfS, PHE, Nuvia, AWE, SCK•CEN, SURO, CREAL, IC)
- **6 Countries** (France, UK, Germany, Belgium, Czech Republic, Spain)
- **Coordination:** IRSN (**Dominique LAURIER**)
- Duration: 18 months – 1 July 2013 → **31 Dec 2014**
- **Funded by DoReMi**



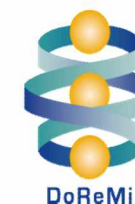
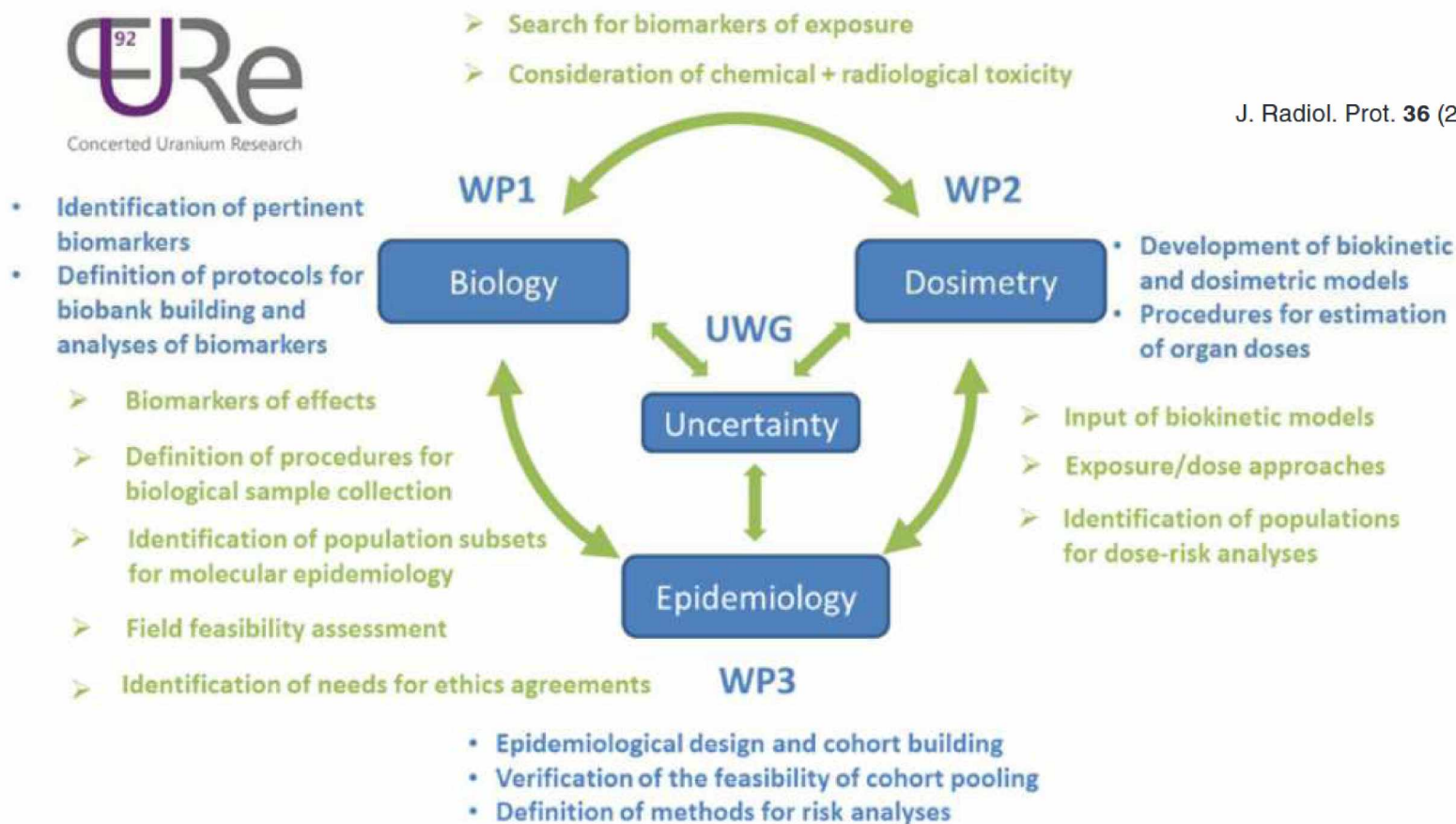
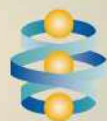
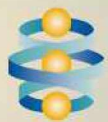


Figure 2. Work packages (WPs) of the CURE project, tasks and interactions between main WPs and the Uncertainty Working Group (UWG).



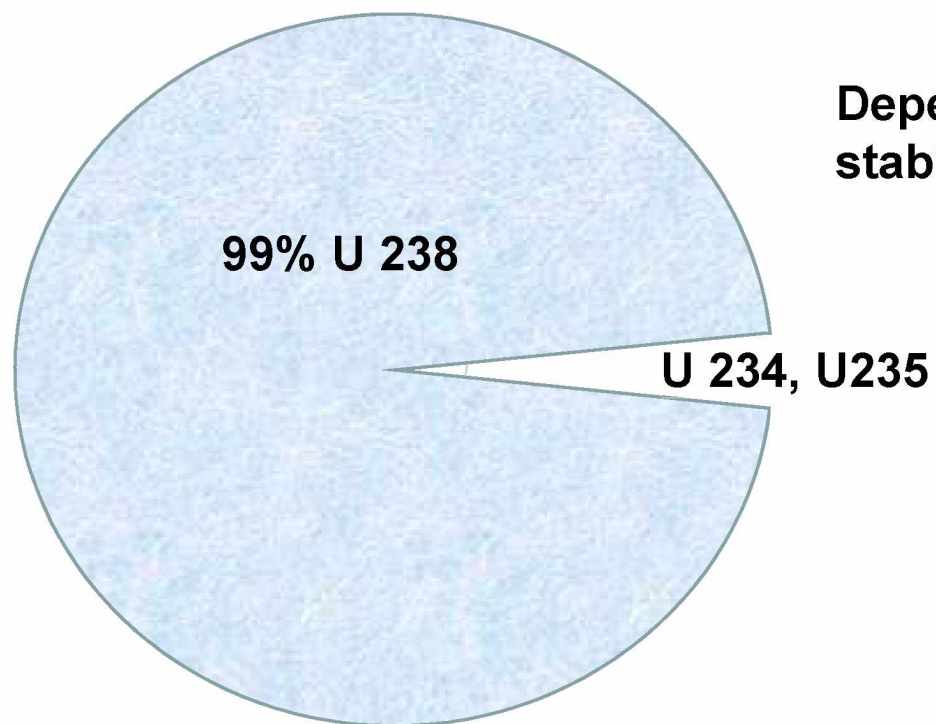
Natural Uranium

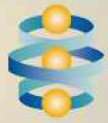
Solubility and Bioavailability:

Radiotoxicity:

**Dependent on
chemical compound**

**Dependent on
stability of isotope**



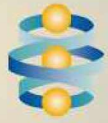


	Bioaccumulation dependent on chemical form (solubility) and intake path Most of the uranium is not absorbed	
Exposure	e.g. U238 Drinking water	e.g. U238 Inhalation
Ingestion	Skeleton	Lung tissue
Inhalation	Kidney	Lymphnodes
	Liver	Skeleton
Skin contact	Soft tissue	Kidney
		Liver

Bioeffects: chemical toxicity/radiological toxicity, ratio dependent on isotope

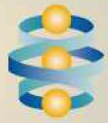
Potentially associated adverse outcomes :

Lung and kidney cancer, nephrotoxicity, respiratory diseases, impaired bone formation
Brain, Cardiovascular effects, hematopoietic system



Open Questions for human health

- Distinction between chemical and radiological toxicity – are there different molecular initiating events and effects?
- Chronic low dose exposure – exposure estimation? Biological and health effects?
- Effects on children and unborn child?
- Combined exposure situations

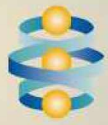


How can radiobiology help to identify and assess health risks (Pernot et al 2012, Hall et al. 2017)

- Refine dosimetry by exposure markers
- Identify vulnerable groups (early onset, stronger reaction,....)
- Identify Adverse Outcome Pathways by early and late effect (disease) markers:

candidate approach and OMIC approach

- Development of biological based models opposed to the sole use of mathematical models of epidemiological data that are primarily driven by medium/high doses

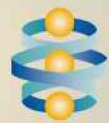


A valid study protocol needs:

- Identification of biomarkers relevant for Molecular initiating (MIE) key biological events (KE) on suspected adverse outcome
- Identification of biospecimen and standardization and harmonization of collection – dependent on envisaged biomarker
- Set up of questionnaire to interpret biomarker results:
 - Identify potential confounders
- Inclusion of medical tests

TABLE 3
List of Proposed Biomarkers to be Investigated in Uranium-Exposed People (Uranyl Nitrate)

Organ or molecular targets	Effects	Biomarkers	Sampling specimen	Justification of biomarker	Biomarker measurement technics	Refs.
DNA	Genomic instability, chromosomal aberration	Dicentrics, translocations, micronuclei	Blood	Induced by ionizing radiations	Cytogenetic conventional or molecular	(98, 102, 107, 132)
Kidney	Dysfunction; impairment of tubules or glomeruli and tubules; reabsorption cardiorenal syndrome	Creatinine, urea, electrolytes, glucose KIM-1, NGAL, RBP cystatin-C, B2M, renin, ACE	Urine and plasma	Uranium storage and suspected target effect; specific and sensitive markers of kidney impairment	Immunoassay multiplex	(162–165)
Lung	Damage (COPD) or lung cancer	CC16; SHOX2, SEPT9	Blood, sputum	Uranium entry and deposition by inhalation	qPCR, immunoassay	(157, 160, 161)
Bone	Resorption defect; osteoporosis, osteosarcoma	CTX, ALP, vitamin D	plasma	Uranium storage at long term	Immunoassay	(76, 136)
Central nervous system	Neurodegeneration; cognitive impairment	S100 β , annexin A5, transthyretin, clusterin, NT-proBNP, Beta-amyloid, neuromediators (DA, NA, 5-HT and catabolites)	Plasma	Uranium cognitive effects in animals	Immunoassay, multiplex	(177, 179)
Heart/endothelium of vessels	CVD; inflammation/endothelial dysfunction	BNP, NT-ProBNP, CK-Mb, CXCL6, troponin I; miRNA; sICAM1, sVCAM1, P-selectin; APO A1, AII, B, CII, CIII, Apo E, TNF, IL-6, MCP1, CRP	Plasma	Suspected direct or indirect effect through kidney; suggestive human data	Immunoassay, multiplex	(167, 168, 171–173)
Hematopoietic system	Leukemia-specific translocations	Chimeric mRNA	Blood	Uranium deposition in bone	qPCR, nCounter	(187–190)
OMICS	Systemic alteration; RNA modification; refine exposure?	Transcriptomics, proteomics; metabolomics	Urine, plasma	To identify unknown markers of exposure or effect	LC-MS, SAGE, SELDI-TOF	(88, 89, 191, 192)



Development of sampling strategies – including clinical tests, questionnaires and high quality samples

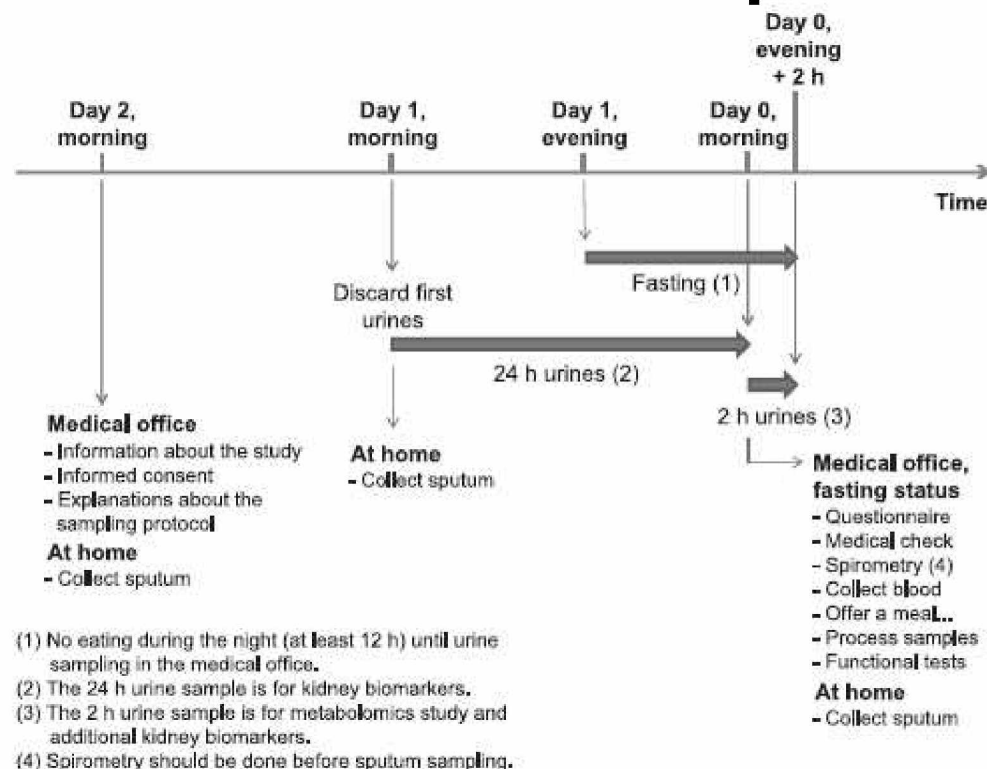
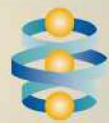
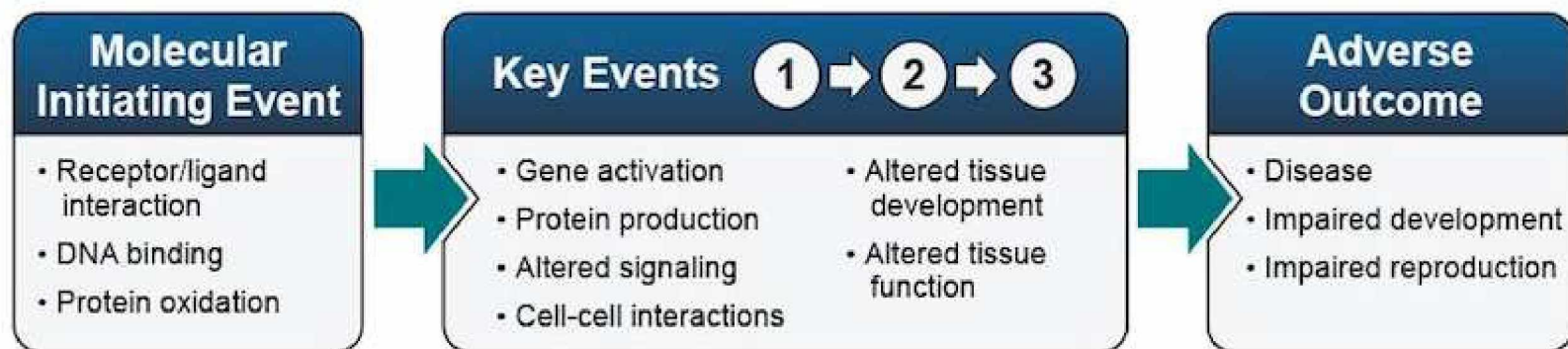


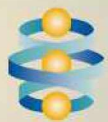
FIG. 1. Schematic representation of sampling time table. The entire process of biological sample collection and medical examinations will last two days for each study participant. To avoid the influence on the biomarkers by, e.g., current infections, clear exclusion criteria must be defined before participants arrive at the collection center. Day 0 represents the day of medical checkup and blood puncture. Day 1 and 2 represent the days before medical checkup, respectively.





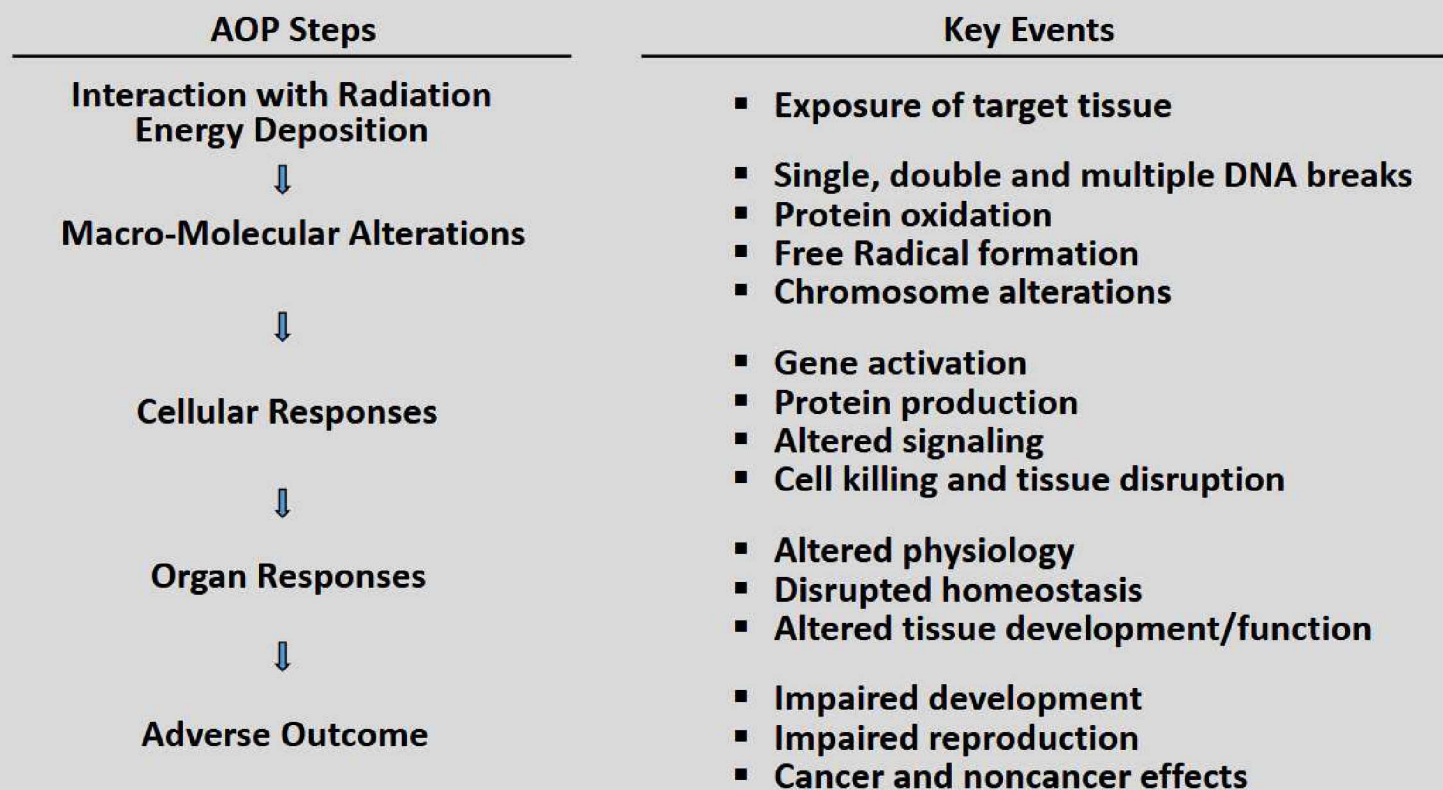
Adverse Outcome Pathways – A strategy for toxicological risk assessment

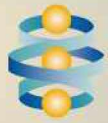




Preston, J.; NAS/NRC 2014

Schematic representation of an adverse outcome pathway (AOP) for ionizing radiation-induced cancer and non-cancer diseases showing each step along the proposed pathway and the linked key events.

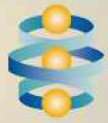




AOPs are particularly designed to allow integration of information spanning from the molecular level, over sub-cellular, cellular and organ levels to the AO at the level of entire organisms

AOPs as such cannot be used for risk assessment UNLESS they are quantitative (qAOPs), but even if only qualitative they describe a potential hazard, given that a specific compound at the concentration, which is reached at a relevant site, triggers the Molecular Initiating Event that activates the AOP. Relevant for chronical low/ very low dose exposure

AOPs are not systems biology models.



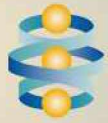
Conclusions from the CURE study:

- A full molecular epidemiology protocol was developed, including a questionnaire and other means of data collection to measure potential confounding factors.
- Such an approach will be useful:
 - 1) to determine the changes in biomarkers which may be attributed to uranium (chemical/radiological action)
 - 2) to better appreciate the relative influence of uranium versus other stressors on these biomarker changes, as part of AOP leading to chronic diseases.
 - 3) Adverse Outcome “hypothetical diseases and their development” can subsequently be further studied in classical epidemiological approaches (at higher doses, e.g. : iPAUW*) and through well-justified radiobiological experiments



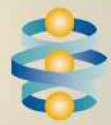
will consolidate elements to draw inferences on risk following exposures to very low doses





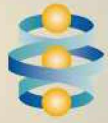
Conclusions: How can radiobiology/molecular epidemiology assist risk quantification

- The continued integration of radiation biology and epidemiology remains an important area for future research, with radiation biology providing the mechanistic structure (**key events in response to chronic/low/very low doses**) for how epidemiological outcomes may be explained.
- But also to **provide ideas for adverse outcomes**, that can be investigated in (molecular) epidemiological projects – biomarker changes and risk inferences can also be provided by large non radiation-cohorts. “Meet-in the middle” approach.
- Development of fully informative biologically based dose–response (BBDR) models to estimate effects at low doses and low dose rates
- Analysis of uncertainty and reduction of uncertainty is critical to the development of risk estimates at low doses:
 - Dose uncertainty
 - Latency period (time between exposure and diagnosis of disease)
 - Shape of the dose responses
 - Dependences on modifying factors related to sex, age and time
 - Disease misdiagnosis



ACKNOWLEDGMENTS

The CURE Project was supported by the European Commission FP7 DoReMi Network of Excellence (grant no. 249689)



DoReMi Integrating Low Dose Research

Thanks for your attention!

